

VI. "The Glucoside Constitution of Proteid Matter." By
F. W. PAVY, M.D., F.R.S. Received May 20, 1893.

(Abstract.)

For a long time I have adopted a process for separating the glycogen of the liver consisting in boiling with potash, pouring into alcohol, and collecting the precipitate. For the purpose of estimation, the precipitated glycogen was converted by means of sulphuric acid into glucose, the determination of which gave the information required. I afterwards applied the process to blood, and the various organs and tissues of the body, with the result that a more or less notable amount of cupric oxide reducing product was obtained, which I at the time looked upon as taking origin, as in the case of the liver, from glycogen.

When small quantities were operated upon for quantitative analysis, I experienced no difficulty in obtaining the product looked for. When large quantities, however, were taken for the purpose of collecting it and studying its properties, I invariably failed to obtain anything like the amount that ought to have been yielded according to the indications afforded by the quantitative analysis conducted. These attempts were undertaken at different times upon blood, eggs, and the spleen. It was obvious there was something connected with the extraction with which I was not acquainted, and it was through prosecuting inquiry to clear up the discrepancy that I came across the knowledge revealed in this communication.

Detailed particulars are given in the communication of experiments with various animal and vegetable proteid matters, showing that the amount of cupric oxide reducing product obtained after the application of the potash process varied through the instrumentality of the potash. If free glycogen or starch had been the source of it, the treatment with potash should have produced no effect beyond dissolving the associated nitrogenous matter and placing it in a position to be separable by the agency of the alcohol, and no difference should have resulted from varying the strength of the alkali or the length of time of contact. The results obtained led to the conclusion that the cupric oxide reducing product must have taken origin from some other source than free glycogen or starch, and pointed to its having been derived from a cleavage of the proteid molecule.

The steps of procedure employed for obtaining the product susceptible of conversion into a cupric oxide reducing body (sugar) from proteid matter are described at length, and an account given of the difficulties which had led to a want of success in my early endeavours to collect the body in quantity. With the knowledge

that is now possessed, no difficulty is experienced in obtaining it in any amount that may be required for the purpose of examination or experiment. Coagulated egg-albumin, purified by water, was selected as the most suitable representative form of proteid to serve for yielding the product.

The properties of the material obtained are as follows :—

In the dried state it forms a hard glassy resinoid mass.

It is readily soluble in water, giving a clear solution.

It yields no coloration with iodine.

It possesses no CuO reducing power.

Strong alcohol precipitates it. Spirit of 85 to 90 per cent. strength precipitates it in great part, if not completely, as an adherent tenacious gummy mass, from which the alcohol may be decanted off, and which may be afterwards worked up to form a sticky material. Absolute alcohol, used freely, throws it down as a fine white precipitate with no tendency to coalesce to a gummy mass. Upon the gummy precipitate absolute alcohol exerts a dehydrating action, causing it to harden and assume a crumbled, in place of a cohesive, state. With spirit below 85 per cent. strength, precipitation becomes more and more incomplete, and the precipitate produced by the weaker kind of spirit assumes a loose or non-adherent form. The incomplete precipitation with weak strengths is readily betrayed by the further precipitation which occurs upon adding more alcohol to the supernatant spirit.

In its physical characters, it presents a resemblance to Landwehr's "animal gum." Chemically, it also resembles Landwehr's "animal gum," in forming a copper compound on being treated with cupric sulphate and caustic potash, from which it is susceptible of recovery by the agency of nitric acid and subsequent precipitation with alcohol.

I have applied Landwehr's process to the product under consideration derived from the action of potash upon albumin, and have found that it behaves throughout like Landwehr's "animal gum," and similarly yields a CuO reducing body. Moreover, I have found that this CuO reducing body gives, with phenylhydrazine, needle crystals of glucosazone, of which I have obtained micro-photographs.

The product, it may be finally remarked, possesses the property of diffusibility, a character in which it differs from the amylose carbohydrates—starch, glycogen, dextrin. I have been unable to find any statement about the diffusibility or otherwise of "animal gum," but it is described as being a constant constituent of urine, which may be regarded as indicative of its being of a diffusible nature.

The CuO Reducing Product.—By the action of mineral acids, the first-formed product, of which I have been speaking, undergoes con-

version into a CuO reducing material. For effecting this conversion I have for years past been in the habit of using 2 per cent. sulphuric acid. As I have said, my impression originally was that the product consisted of glycogen, and from observations upon the conversion of starch and glycogen into glucose, I had formed the opinion that sulphuric acid of the strength named best met the requirements. Boiling for about an hour and a half with an inverted condenser was resorted to, or the autoclave was used, half-an-hour's exposure to a temperature of 150° C. producing an equivalent effect, the results given by the two methods being practically identical. Where the object has been a quantitative analysis, the acid has been subsequently neutralised with potash, the sulphate formed not occasioning any inconvenience; but should it be desired to collect the reducing product, the acid must be removed by precipitation, best effected by the agency of barium carbonate; the filtrate, after evaporation to dryness on the water-bath, yields the material in the state desired.

I had been led by certain circumstances to consider that the reducing product derived from the action of the 2 per cent. acid did not consist of glucose, and had unsuccessfully tried, by more extended periods of boiling, to get it carried further. Subsequently I renewed my efforts in this direction. In experimenting with cellulose, I had found that but very slight action was produced by 2 per cent. acid, while considerable effect followed the use of acid of 10 per cent. strength, and it occurred to me that the body I was dealing with might behave similarly. Trying 10 per cent. acid, I found that the reducing power of the product became very nearly doubled, being raised in the proportion of from between 50 and 60 to 100. I next tried 50 per cent. acid, allowed to remain in contact with the product for one to three days, then diluted to 20 per cent., and boiled. The result stood about the same as after direct boiling with 10 per cent. acid. After 15 per cent. acid, also, a like result was obtained. As yet I have failed to carry the body to a higher stage of CuO reducing power than that produced by 10 per cent. acid, but I am still led to think that the glucose stage has not yet been reached. The effect of raising the reducing power is to give a semblance of a corresponding increase of material.

The following are the characters of the product obtained after the separation of the sulphuric acid by barium carbonate and evaporation of the filtered liquid to dryness:—

It presents the appearance of a sugary extractive, and has a pronounced baked-sugar odour.

It is very soluble in water, only slightly so in absolute alcohol, but considerably so in spirit of about 90 per cent. strength.

It is readily diffusible.

Boiled with caustic potash (Moore's test), the solution darkens.

It dissolves hydrated copper oxide in presence of excess of potash (Trommer's test), without producing a biuret reaction; at least, specimens are often procurable of which this can be said.

With Fehling's solution, it gives a strong and neat reaction, the reduced copper oxide falling as a dense red precipitate.

Heated on the water-bath for two or three hours with phenylhydrazine and acetic acid, it yields, on cooling, a crystalline osazone in the form of needles clustered into sheaves or brushes, or radiating in dense round masses. Often the composite character of these round masses is only to be seen by close inspection of the circumference. Whilst a certain type prevails, variations within limits are noticeable, and sometimes an approach to a spike constitution is observable. The crystals are soluble in alcohol, from which they may be recrystallised.

It gives with benzoyl chloride an insoluble compound, in accord with the behaviour of carbohydrates.

With α -naphthol and excess of strong sulphuric acid, it behaves like sugar in giving a deep-violet colour, and leading on dilution to the formation of a violet-blue precipitate soluble in alcohol, ether, and caustic potash, with the production of yellow solutions, but insoluble in hydrochloric acid, a character by which, according to Molisch, the precipitate produced from sugar is distinguishable from that derived from peptone and various albuminous bodies.

With thymol and excess of strong sulphuric acid, it again behaves like sugar, giving a deep-red coloration, followed, on dilution, by the production of a carmine-red precipitate, soluble in alcohol, ether, and caustic potash, with formation of pale-yellow solutions, and in ammonia with formation of a bright-yellow solution. The precipitate, as in the case of the α -naphthol test, is found to possess the character of insolubility in hydrochloric acid.

It is susceptible of being thrown down in combination with oxide of lead, by the employment of lead acetate and ammonia, and is afterwards recoverable from the compound. From the sugar thus recovered, the usual reaction is obtainable with Fehling's solution (provided the ammonia has been fully removed); and also the crystalline osazone with phenylhydrazine, of which micro-photographs are furnished.

From this assemblage of positive characters, it appears to me there can be no doubt that the CuO reducing body obtainable from proteid matter consists of sugar. I should state, however, that I have not yet obtained it in a form to prove fermentable; and Dr. Sheridan Lea, who kindly examined it with the polarimeter, failed to notice any rotation of which he could speak with certainty. He adds there was, if anything, a tendency to lævorotation, but not amounting to more than 0.1° .

In addition to the evidence derivable from the positive characters mentioned as possessed by the CuO reducing product, there is the corroborative evidence to be taken into account supplied by the characters of agreement observable between my primary non-reducing product and Landwehr's "animal gum." As already shown, the product in question can be thrown down, precisely like "animal gum," in combination with copper oxide, and is afterwards recoverable and convertible into a CuO reducing body, which yields with phenylhydrazine a crystalline osazone. The micro-photos. supplied show two kinds of crystals, one consisting distinctly of the long needles of glucosazone. In its ready mode of separating out, the osazone was observed to agree with that derived from glucose. A 10 per cent. strength of sulphuric acid was employed in obtaining the CuO reducing product operated upon.

Presents, June 8, 1893.

Transactions.

- Berlin :—Gesellschaft für Erdkunde. Zeitschrift. Band XXVIII.
No. 1. 8vo. *Berlin* 1893. The Society.
- Brussels :—Société Malacologique. Annales. Tome XV. Fasc. 2.
Tome XXVI. 8vo. *Bruxelles*; Procès-Verbal. Juillet, 1891—
Septembre, 1892. 8vo. *Bruxelles*. The Society.
- Bucharest :—Societății de Științe Fizice. Buletinul. Anul II.
Nos. 3—4. 8vo. *București* 1893. The Society.
- Cambridge, Mass. :—American Academy of Arts and Sciences.
Memoirs. Vol. XII. No. 1. 4to. *Cambridge* 1893. The Academy.
- Harvard University. Bulletin. Vol. VII. No. 3. 8vo. 1893. The University.
- Museum of Comparative Zoölogy. Bulletin. Vol. XVI. No. 12.
Vol. XXIV. No. 3. 8vo. *Cambridge*, 1893. The Museum.
- Cracow :—Académie des Sciences. Bulletin International. Mars,
Avril, 1893. 8vo. *Cracovie* 1893. The Academy.
- Kew :—Royal Gardens. Bulletin of Miscellaneous Information.
Nos. 74—75. 8vo. *London* 1893. The Director.
- London :—British Astronomical Association. Journal. Vol. III.
Nos. 6—7. 8vo. *London* 1893. The Association.
- East India Association. Journal. Vol. XXV. Nos. 4—5. 8vo.
London 1893. The Association.
- Geologists' Association. Proceedings. Vol. XIII. Part 2. 8vo.
London 1893. The Association.
- Institute of Brewing. Transactions. Vol. VI. No. 6. 8vo.
London 1893. The Institute.